Texturized Dairy Proteins

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ABSTRACT: Dairy proteins are amenable to structural modifications induced by high temperature, shear, and moisture; in particular, whey proteins can change conformation to new unfolded states. The change in protein state is a basis for creating new foods. The dairy products, nonfat dried milk (NDM), whey protein concentrate (WPC), and whey protein isolate (WPI) were modified using a twin-screw extruder at melt temperatures of 50, 75, and 100 °C, and moistures ranging from 20 to 70 wt%. Viscoelasticity and solubility measurements showed that extrusion temperature was a more significant ($P < 0.05$) change factor than moisture content. The degree of texturization, or change in protein state, was characterized by solubility ($R^2 = 0.98$). The consistency of the extruded dairy protein ranged from rigid (2500 N) to soft (2.7 N). Extruding at or above 75 °C resulted in increased peak force for WPC (138 to 2500 N) and WPI (2.7 to 147.1 N). NDM was marginally texturized; the presence of lactose interfered with its texturization. WPI products extruded at 50 °C were not texturized; their solubility values ranged from 71.8% to 92.6%. A wide possibility exists for creating new foods with texturized dairy proteins due to the extensive range of states achievable. Dairy proteins can be used to boost the protein content in puffed snacks made from corn meal, but unmodified, they bind water and form doughy pastes with starch. To minimize the water binding property of dairy proteins, WPI, or WPC, or NDM were modified by extrusion processing. Extrusion temperature conditions were adjusted to 50, 75, or 100 °C, sufficient to change the structure of the dairy proteins, but not destroy them. Extrusion modified the structures of these dairy proteins for ease of use in starchy foods to boost nutrient levels.

Practical Application: Dairy proteins can be used to boost the protein content in puffed snacks made from corn meal, but unmodified, they bind water and form doughy pastes with starch. To minimize the water binding property of dairy proteins, whey protein isolate, whey protein concentrate, or nonfat dried milk were modified by extrusion processing. Extrusion temperature conditions were adjusted to 50, 75, or 100 °C, sufficient to change the structure of the dairy proteins, but not destroy them. Extrusion modified the structures of these dairy proteins for ease of use in starchy foods to boost nutrient levels.

Keywords: dairy proteins, extrusion, nonfat dried milk, texturization, whey protein concentrate, whey protein isolates

Introduction

Texturization is a process that uses mechanical shear to unravel the globular structure of native proteins, and can be accompanied by breakage of intra-molecular bonds, and re-alignment of disulfide bonds, with heat or pressure as requisites (Bhattarcharya and Padmanabhan 1999). An extruder is one device used to accomplish texturization of proteins through the actions of its internal rotating screws pressing the protein against the fixed heated barrel walls, and forcing the molten protein mass through a restriction die that aligns the protein mass in the direction of rotational flow. Extrusion texturization of proteins can occur at temperatures ranging from 50 to 100 °C and at short residence times (< 2 min), far below the range of thermochemical denaturation conditions for other proteins, for instance, wheat glutens in dry heat from about 200 to 215 °C for 72 min (Friedman and others 1987), or collagen in moist heat below 120 °C for 30 min (Meyer and others 2005).

Texturization is not measured directly, but it is inferred from the degree of denaturation or insolubilization of proteins determined by the difference in rates of moisture uptake between the native protein and the (extruded) texturized protein (Kilara 1984), or by a dye binding assay (Bradford 1976). Proteins are denatured when the native globular conformations are modified or unfolded as a result of some physical process, for example, spray drying, where there is no change in the primary structure, bond cleavages, or functional properties associated with denaturation (Bhattarcharya and Padmanabhan 1999). Denaturation of proteins can be measured by determining changes in heat capacity, but it is more practical to determine differences in solubility after physical treatment, and by the amount of insoluble fractions (Kilara 1984). Texturized proteins absorb water at different rates and it is assumed that the rates of water absorption are related to the degree of texturization; therefore, the insolubility test for denaturation is sometimes used as substitute for measuring texturization directly. Solubility of proteins is affected by their surface hydrophobicity, which is directly related to the extent of protein–protein interactions, an intrinsic property of the denatured state of the proteins (Damodaran 1988; Vojdani 1996).

Manufacturing of expanded snacks using nontexturized (unmodified) whey proteins in large amounts is only marginally successful (Singh and others 1991), but if proteins are texturized or modified along with, or prior to adding them to the starch matrix, a better expanded functionally improved product can be created leading to improved texture (Mohammed and others 2000). Whey proteins can be modified by chemical reagents, heat, or shear in the extruder (Kim and Maga 1987). Using chemical treatment alone, the reactive groups of the amino acids can be exposed, resulting in changes in the noncovalent forces that influence conformation

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such as van der Waals forces, electrostatic interactions, hydrophobic interactions, and hydrogen bonding (Kester and Richardson 1983). Heat and shear alters the conformation of proteins through partial denaturation of the protein molecules that exposes groups that are normally concealed in the folded native protein (Kim and Maga 1987).

To improve the interaction of whey proteins with other food components such as starches, flours, and nondairy proteins, different methods have been explored principally to increase extrudate expansion. For example, to directly expand high protein corn meal containing 30 wt% whey protein concentrate (WPC), extreme extrusion process conditions of high shear and low moisture were used (Onwulata and others 2001). In a similar process, an expanded extrudate was made using low temperature (< 100 °C) and low shear with supercritical CO_2 (SCFX) extrusion (Rizvi and Mulvaney 1993). The range of use for unmodified whey proteins in puffed extrudates can be extended with difficulty in amounts greater than 10 wt% (Kim and Maga 1987; Onwulata and others 1998).

Recent efforts have focused on expanding the functionality of whey protein products for use in other products without using extreme extrusion processing conditions. To accomplish this, a pretexturization step is necessary to modify the proteins by chemical, enzymatic, or physical means, for enhanced food functionality such as improved solubility. Using physical means primarily, new surface structures effects were created for a range of whey proteins broadening their functionality (Onwulata and others 2003; Onwulata and Tomasula 2004). Extrusion texturization was used to create fibrous structures used as a basis for whey-protein-based meat extenders (Hale and others 2002; Walsh and Carpenter 2003). Whey protein-fortified expanded products (Onwulata and others 1998; Onwulata and others 2001; Walsh and Carpenter 2003), and cold-setting gels (Manoi and Rizvi 2009) were created by adjusting texturization conditions.

It is known that direct whey texturization intensifies protein-protein networks and improves the matrix network patterns resulting in increased shear modulus (Tunick and Onwulata 2006). Texturization of different whey protein fractions using the SCFX process over a wide range of temperatures below 90 °C transformed the whey proteins into cold-setting gels (Manoi and Rizvi 2008). The goal of this research was to determine the effects of temperature and moisture of extrusion texturization on the physical properties of nonfat dried milk (NDM), WPC, and whey protein isolate (WPI).

Materials and Methods

NDM was obtained from Land O'Lakes (Carlisle, Pa., U.S.A.). WPC containing at least 80% protein (WPC80) was purchased from Davisco Foods Intl. Inc. (Le Sueur, Minn., U.S.A.). WPI (Provon 190) was purchased from Glanbia Ingredients Inc. (Monroe, Wis., U.S.A.). The percentages of components of the starting materials, according to the manufacturers' specifications are: WPI, moisture 2.8%, protein 89.6%, fat 2.5, ash 3.3%, carbohydrate by difference; WPC80, moisture 4.8%, protein 77.6%, fat 7, ash 3%, carbohydrate by difference; NDM, moisture 3.2%, protein 36.2%, fat 0.8%, ash 7.9%, carbohydrate by difference. The milk powders were extruded in a model ZSK30 twin-screw extruder (Krupp, Werner & Pfleiderer Co., Ramsey, N.J., U.S.A.) consisting of 9 barrel zones, each with individual temperature control. The screw elements were selected to provide low shear at 300 rpm; the screw profile was described by Onwulata and others (1998). Feed was conveyed into the extruder with a Series 6300 digital feeder, type T-35 twin screw volumetric feeder (K−tron Corp., Pitman, N.J., U.S.A.). The feed screw speed was set at 600 rpm, corresponding to a rate of 3.50 kg/h. Water was added into the extruder with an electromagnetic dosing pump (Milton Roy, Acton, Mass., U.S.A.). At different water pump settings ranging from 20 to 70 (1.3 to 6 L/h), water input rates were: 1.556 (pump set) − 9. Samples were collected after 25 min of processing, freeze-dried overnight in a VirTis Freeze Mobile 12XL Research Scale Freeze Dryer (Gardiner, N.Y., U.S.A.), and stored at 4.4 °C until analyzed. The experiments were performed in triplicate.

Analysis of variance was used to identify differences in physical properties at various processing conditions. Principal Components Analyses of dairy proteins and regression analyses of measured attributes and correlation coefficients were calculated using the Statistical Analysis System (SAS) package (SAS Inst. Inc, Cary, N.C., U.S.A.) in all cases. All statistical hypothesis testing was performed at $P = 0.05$ significance level.

Moisture was determined by the AOAC Official Method 925.10. Extrudate samples weighing approximately 1.5 g were dried in a vacuum oven at 100 °C overnight (AOAC 2000).

Water absorption index (WAI) was determined by modifying the methods reported by Jin and others (1995). Extrudates were ground and sifted through a 210-micron sieve, 1 g of extrudate (± 0.005 g), was placed in a centrifuge tube and 10 mL of distilled water was added. After standing for 15 min (shaking every 5 min), the extrudates were centrifuged for 15 min at 1000 × *g* (Econospin Model, Sorvall Instruments, Wilmington, Del., U.S.A.); the supernatant was decanted: and, the weight gain in the gel was recorded. WAI was calculated as the weight gain of the extrudate on a dry weight basis.

Peak force was determined using a texture analyzer TA-XT2 (Stable Micro Systems, Surrey, England), with a 500 N load cell and a Warner–Bratzler shear cell (1 mm thick blade). The samples were analyzed at a cross head speed of 0.2 mm/s. Peak force (N) was determined as the maximum force required to break the extruded milk protein ribbons (approximately 30 mm wide and 4 mm thick) in the shear cell. Data reported are averages of 10 specimens.

A HunterLab ColorQuest XE dual xenon flash spectrophotometer (400 to 700 nm wavelengths) with optical sensors was used to acquire color data (Hunter Associates Lab., Reston, Va., U.S.A.). Specimens were analyzed in the reflectance mode from an integrated tristimulus data acquired by diode array and converted into color values. Hunter *L*, *a*, *b* values were used to calculate total color difference (ΔE), defined as the square root of ($\Delta L^2 + \Delta a^2 + \Delta b^2$); where the differences (Δ) were with respect to standard reference tile values ($L = 98.34$; $a = -0.21$; $b = 0.19$). Color determinations were made on stored samples 24 h after extrusion. Four readings were taken for each sample.

Protein was determined with 0.2 g extrudate analyzed with the LECO Protein Analyzer Model FP2000 (LECO Corp., St. Joseph, Mich., U.S.A.). Percent protein was calculated with the Nitrogen conversion factor 6.38 for whey protein.

Dye binding protein solubility assay was conducted using the Bradford method (Bradford 1976). Protein insolubility (denaturation) was determined with 1 g ground freeze-dried specimen mixed with 90 mL deionized water. The protein suspension was stirred (125 rpm) at pH 7 for 2 h. The suspension was centrifuged for 20 min, and the supernatant was freeze dried overnight. The LECO Protein Analyzer Model FP2000 (LECO Corp.) was used to analyze the solids from the supernatant for protein content. Protein insolubility (denaturation) was calculated as described by Kilara (1984) as: (% total protein − % soluble protein = % insoluble [denatured]).

Foam volume and stability of extruded proteins were determined using the method described by Vaghela and Kilara (1996) by heating 2.3 g samples mixed with 35 mL deionized water to 60 °C for 15 min. The slurry was then whipped for 15 s in Waring

Lab Micronizer FPC70 (Waring Products Div., New Hartford, Conn., U.S.A.), then transferred to a 100 mL graduated cylinder where the foam volume was read initially, and then every 5 min for 1 h. Foam stability (foam capacity at specific time) over the 1 h period was calculated.

Protein digestibility was determined with 10 mL extrudate sample dissolved in distilled water; the pH was adjusted to 8 with 0.1 N NaOH or HCl. One milliliter of freshly prepared enzyme stock solution (1.6 mg/mL trypsin, 3.1 mg/mL Chymotrypsin, and 1.3 mg/mL aminopeptidase) was added to the protein suspension at 37 °C. The pH after 10 min was recorded with a portable pH meter (IQ Scientific Instruments Inc., San Diego, Calif., U.S.A.), and a Tris/HCl buffer containing 2% SDS (w/v) and 0.1% mercaptoethanol (v/v) was added to the protein solution, which was immediately heated to 90 °C to terminate the enzymatic reaction. Samples were then analyzed by quantitative gel electrophoresis. The percent (%) protein digestibility was calculated by the following equation: % digestibility = 210.46 – 18.10(X); where X is the pH (Singh and Creamer 1993).

The microstructure of the extruded products was visualized by confocal fluorescence imaging and scanning electron microscopy. Segments of extruded ribbons were excised with a stainless steel razor blade and immersed in 2.5% glutaraldehyde buffered with 0.1 M imidazole buffer at pH 7.2 and stored in sealed vials for further processing. For confocal fluorescence, a cross section of an extruded ribbon in the glutaraldehyde fixative solution was mounted onto the coverslip area of a culture dish (MatTek Inc., Ashland, Mass., U.S.A.) and fluorescence images were collected with a model IRBE inverted microscope equipped with a 20 × lens and a confocal scanner head. Fluorescence was excited with the 488-nm line of an Argon laser and autofluorescence from whey protein was collected in an emission channel extending from 500 to 540 nm. Extended focus images from 20 to 30 μm deep volumes were projected as digital image files for comparisons of microscopic structure.

For scanning electron microscopy, segments of extruded ribbons in glutaraldehyde fixative solution were washed in imidazole buffer, dehydrated in graded ethanol solutions, frozen and fractured in liquid nitrogen and critically point dried from carbon dioxide. After mounting and sputter coating with gold, fracture faces of the ribbon segments were visualized in a model Quanta 200 (FEI Co. Inc., Hillsboro, Oreg., U.S.A.) operating in the high vacuum, secondary electron imaging mode.

For SDS PAGE assay, samples were vortexed and dissolved in 20 mM TRIS/HCl, 5 mM EDTA, 2.5% SDS with and without 5% 2-mercaptoethanol at pH = 8 then heated in boiling water for 2 min. Bromophenol blue was added to about 0.1% concentration. The samples were at 2 mg/mL concentration. Phast gels (Amersham Pharmaica Biotech, Uppsala, Sweden) were run according to the procedures given by the manufacturer for SDS 20% homogeneous gels. The 6 lane (4 uL per lane) sample applicators were used. Protein staining used was the Coomassie blue procedure given by the manufacturer (Farrell and others 1998).

Small amplitude oscillatory shear analyses were conducted in triplicate at room temperature using an AR-2000 rheometer (TA Instruments, New Castle, Del., U.S.A.) with parallel aluminum plates. Semi-solid specimens were cut into disks measuring 25 mm in diameter and 2.5 to 4 mm thick; extrudates were used as is because of high moisture content. Strain sweeps were performed to determine the linear viscoelastic range of the sample, which was always between 0.2% and 0.8% strain. Frequency sweeps were then run from 1 to 100 rad/s. Values for elastic or storage modulus (G′) and viscous or loss modulus (G″) were obtained at 10 rad/s. Loss modulus, the ratio of G″ to G′, was also calculated. Linear regressions and standard deviations were calculated by the SAS package (Tunick and Onwulata 2006).

Results

The physical properties of extruded NDM with approximately 36% protein are presented in Table 1. Extruding NDM at process temperature conditions ranging from 50 to 100 °C did not result in a significant ($P < 0.05$) change in product (extrudate) moisture content, but within each preset step temperature condition 50, 75, or 100 °C, changing the extrusion moisture input resulted in marginal increases in moisture content and in foam expansion properties. Increasing the water input from 50% to 70% during extrusion texturization of NDM at 75 or 100 °C increased NDM extrudate solubility marginally. Compared to the native nonextruded NDM, the extruded NDM extrudate WAI decreased significantly at all temperature set points. On the aggregate, slightly less water was absorbed by NDM extruded at 75 or 100 °C. Texturizing NDM by extrusion did not change its foaming, color, and protein digestibility properties significantly. However, the peak force needed to break extruded NDM increased significantly under low moisture condition (30%) and at 75 °C. The lower the moisture at a given

Table 1 — Physical properties of texturized nonfat dry milk.

Temp. (°C)	Water pump setting	Moisture (%)	Solubility (%)	WAI (%)	Foam_Exp	Peak_Force (N)	Prot_Dig	ΔE
50	30	43.4	19.5	22.8	93.5	140.3	86.2	65.1
50	40	50.3	18.0	22.7	100.7	138.1	85.9	64.0
50	50	52.0	19.8	22.6	100.7	45.2	85.2	66.4
50	60	52.7	20.0	21.9	115.0	29.5	85.9	66.5
50	70	56.4	20.7	21.7	116.4	14.6	85.1	64.5
75	30	36.9	18.1	21.2	114.3	220.9	85.1	64.4
75	40	43.8	17.6	21.7	114.2	142.2	84.9	61.9
75	50	48.1	21.1	22.0	113.6	50.7	86.2	65.8
75	60	50.8	22.2	22.3	116.4	35.6	85.9	68.5
75	70	54.5	23.0	22.2	116.4	18.4	86.2	67.1
100	30	41.0	17.8	21.8	120.7	76.1	86.2	75.9
100	40	48.0	15.9	21.7	122.6	34.3	86.0	55.1
100	50	54.2	19.7	22.1	117.1	27.0	86.2	62.9
100	60	57.1	24.9	21.2	121.4	17.4	86.4	67.2
100	70	58.9	24.4	20.7	120.4	26.8	86.2	69.0
	R^2	0.98				0.87		

Temp = temperature set points; Water = water set points; WAI = water absorption index; Foam_Exp = foam expansion; Prot_Dig = protein digestibility; and ΔE: color difference. Native Protein Solubility 46.9; R^2: Significant correlations ($P < 0.05$). Mean square error values were: Moisture (5.13), Solubility (0.64), WAI (0.002), Foam_Exp (9.48), Peak_Force (3.92), Prot_Dig (0.26), and ΔE (12.69).

extrusion temperature, the higher the peak force required to break NDM extrudates. Input moisture was highly correlated with peak breaking force ($R^2 = 0.87$) for NDM extrudate, indicating a weakening or softening of the texturized extrudates; the other values were not correlated. Water input was not a significant factor for the texturization of NDM; no significant differences resulted from varying temperature. In particular, solubility, and water absorption of extruded NDM were not changed significantly by extrusion temperatures ranging from 50 to 100 °C. However, increasing moisture from 50% to 70% at any preset temperature resulted in slightly increased solubility, foam expansion, and reduced peak force. Lactose was the predominant component (approximately 62%) in NDM, and could have absorbed most of the water, limiting the amount of water available for protein texturization. This could have overwhelmed the texture forming or crosslinking capacity of the proteins (approximately 36%) present, preventing protein–protein interactions.

The spatial relationships of the preset temperature and moisture variables of NDM associated through the analysis of their principal component eigenvector values for peak force, foaming, and expansion are presented in Figure 1. The principal components analysis (PCA) weighted in the 1st component (63%) by peak force and the 2nd by foaming expansion (29%) formed 1 big cluster with the others scattered in the 4 quadrants of Figure 1 as follows: top left (100 °C/30% moisture and 100 °C/40% moisture) was characterized by high foam expansion and strong peak force; bottom left (75 °C/30% moisture; 75 °C/40% moisture) were mostly moderate to low foaming properties and strong peak forces; among this group, 50 °C/30 moisture exhibited very poor foaming and moderate peak force. The top right quadrant, mostly high temperature (75 and 100 °C) and high moisture (60% to 70%) conditions, represented high foaming but very weak extrudate peak forces. The bottom right quadrant consisted of low and intermediate temperature (50 and 75 °C) and high moisture extrudates (50% to 70%), mostly moderate foaming and peak forces. In general, for NDM extrudate, high temperature and low moisture conditions increased foaming and peak forces (texturization); moderate to low temperatures (50 to 75 °C) and moisture (30% to 40%) suppressed foaming and texturization, resulting in softer and gel-like extrudates.

The physical properties of texturized WPC containing approximately 80% protein (WPC80) are presented in Table 2.

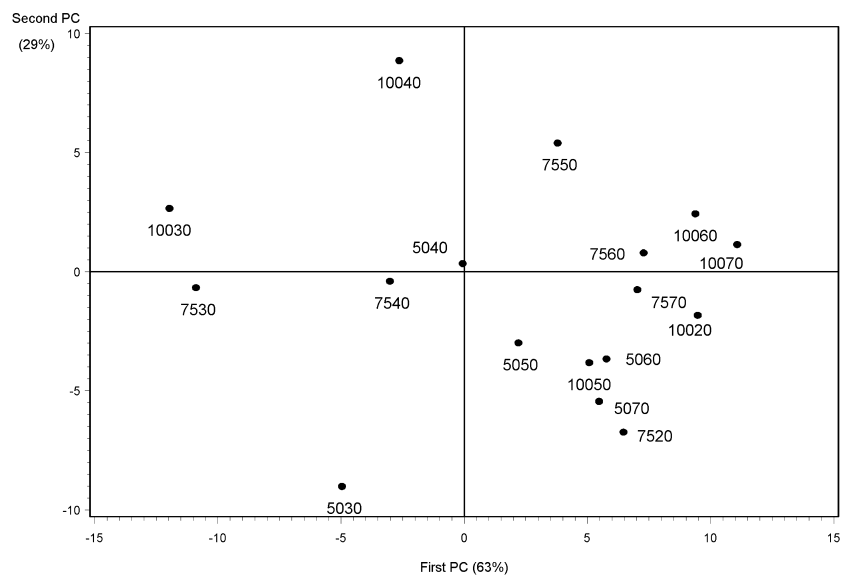

Figure 1 — Spatial arrangement of process temperature and moisture for NDM based on the PCA of peak force of texturization (Component I, accounting for 63%), and foaming (Component II, accounting 29%). Code designation: 5030 (50 °C : 30% moisture); 10030 (100 °C : 30% moisture).

Table 2 — Physical properties of texturized WPC80.

Temp (°C)	Water pump setting	Moisture (%)	Solubility (%)	WAI (%)	Foam_Exp	Peak_Force (N)	Prot_Dig	ΔE
50	20	35.4	64.5	54.1	85.7	1776.4	108.1	85.7
50	30	46.6	65.8	61.6	112.1	2758.9	93.5	87.0
50	40	50.6	65.0	68.9	118.9	448.3	92.7	90.2
50	50	56.8	64.1	69.6	121.8	70.2	91.3	90.0
50	60	53.7	51.6	59.0	129.3	19.0	91.6	90.1
75	20	30.9	3.9	28.3	110.4	1186.8	91.3	84.4
75	30	39.1	7.8	31.4	114.3	487.3	92.0	87.9
75	40	51.0	13.1	26.0	122.1	169.3	92.7	89.5
75	50	54.3	21.7	20.9	117.9	288.4	92.3	89.3
75	60	58.9	28.1	17.0	108.6	108.9	91.9	90.4
100	20	30.5	2.6	29.2	115.7	1108.1	92.0	83.2
100	30	35.6	3.2	31.6	114.3	686.7	91.8	90.0
100	40	46.4	4.6	32.0	116.1	278.1	90.5	91.3
100	50	54.5	7.8	31.5	113.6	382.6	90.0	91.7
100	60	59.5	10.9	29.1	112.1	171.9	89.9	91.4
	R^2	0.91	0.88	0.91				0.87

Temp = temperature set points; Water = water set points; WAI = water absorption index; Foam_Exp = foam expansion; Prot_Dig = protein digestibility; and ΔE: color difference. Native protein solubility 83.8; R^2: Significant correlations ($P < 0.05$). Mean square error values were: Moisture (25.20), Solubility (46.07), WAI (0.21), Foam_Exp (922.9), Peak_Force (7482.3), Prot_Dig (0.16.8), and ΔE (2.41).

Temperature conditions ranging from 50 to 100 °C did not change the aggregate moisture content of extruded WPC80 significantly. High temperatures (75 and 100 °C) reduced solubility and WAI. As moisture content at a given temperature increased, so did the lightness values reflected in increased total color difference (ΔE). Texturized WPC80 was mostly insoluble at 75 °C or above; yet, the peak forces of the extrudates were weaker at high moisture contents ranging from 30 to 60 w%. Texturization of WPC80 was accomplished at higher temperatures ranging from 75 to 100 °C as indicated by low solubility (<35%). Changes in water absorption ($R^2 = 0.91$), water solubility index ($R^2 = 0.87$), WHC ($R^2 = 0.96$), and total color difference ($R^2 = 0.84$) were all highly significantly ($P < 0.05$) correlated with the process preset moisture conditions ($R^2 = 0.91$) indicating that preset moisture conditions influenced texturization of WPC80. Solubility and WAI of texturized WPC80 were significantly reduced at temperature greater than 50 °C and moisture greater than 30%. Because of the small amount of lactose present in WPC80 (approximately 6%) and the large protein content (> 80%), enhanced protein–protein interaction occurred, improving texturization. Increased texturization may be inferred from the low solubility values for WPC80 extruded at 75 or 100 °C. Increased degree of texturization also resulted in decreased WAI, peak force, and digestibility. Protein digestibility and total color difference (ΔE) were not changed significantly.

The spatial PCA distribution of the variables temperature and moisture conditions for extruded WPC80 based on peak force and foam expansion is presented in Figure 2. The PCA weighted in the 1st component (54%) by peak force and the 2nd by foam expansion (38%) fell in 2 big clusters with the other process conditions scattered in the 4 quadrants of Figure 2. The top left quadrant was mostly process conditions of 75 and 100 °C and moisture ranging from 20% to 70% representing moderate foam expansion and high extrudate peak break force. The bottom left quadrant (100 °C/30% moisture and 75 °C/60% moisture) represent low foam expansion and high peak forces. The top right and bottom quadrants form 1 big cluster of weak peak forces and moderate foaming, with only the 50 °C/20% moisture product being the outlier with very low foaming and weak force; essentially, an extrudate that was not texturized. The big cluster to the right, which consisted mostly of extrudates of 50 °C/30, 40, 50, and 60% moisture, and 75 °C/50% moisture products representing moderate to low foaming capacity and very weak force. For WPC80 texturization, temperatures greater than 75 °C resulted in reduced solubility and moisture content greater than 30 wt% showed progressively weaker peak forces and softer gels. Protein digestibility remained high, but total color difference increased, reflecting increasing lightness value.

The physical properties of WPI containing approximately 95% protein are presented in Table 3. Extruding WPI at temperature conditions ranging from 75 to 100 °C and moisture ranging from 20% to 60%, reduced extrudate moisture, solubility, water absorption, and foam expansion significantly, but not the peak force, digestibility or total color difference. The effect of moisture was significant only for solubility. Solubility was very high for 50 °C extrudates and WAI was very low, but as the moisture values increased from 43.8% to 67.6%, solubility decreased from 92.6% to 71.8% showing that at 50 °C high moisture content increased texturization, and reduced solubility. Extrudates produced at 75 and 100 °C were mostly insoluble or texturized, but their WAI values increased. At 75 °C, increasing the moisture content from 20% to 60% increased solubility. At 50 °C extrusion, texturization (reduced solubility) increased with increasing moisture content, but the trend was reversed at 75 and 100 °C where texturization increased with moisture. At these conditions, increasing moisture reduced texturization (higher solubility). Increasing the moisture in WPI extruded above 75 °C reduced texturization as measured by increasing solubility. Extrudate moisture content was highly correlated with the process preset water conditions ($R^2 = 0.98$), water absorption ($R^2 = 0.92$), water solubility index ($R^2 = 0.93$), and WHC ($R^2 = 0.92$) showing that moisture affects the properties of WPI. Also, protein foaming properties such as foam volume ($R^2 = 0.74$), capacity ($R^2 = 0.76$), stability ($R^2 = 0.77$), expansion ($R^2 = 0.75$), and peak force of the extrudates ($R^2 = 0.74$) were more highly correlated with solubility or texturization of WPI with 96% protein, than the others, NDM with 34% and WPC80 with approximately 80% protein.

The spatial distribution of preset temperature and moisture conditions for WPI based on their PCA peak force and foam expansion values are presented in Figure 3. The PCA weighted in the 1st component (73%) by peak force and the 2nd by foam expansion (13%) fell into 3 clusters in the moderate peak force zones of the 4 quadrants, around the strong peak force central axis. For 2 clusters 100

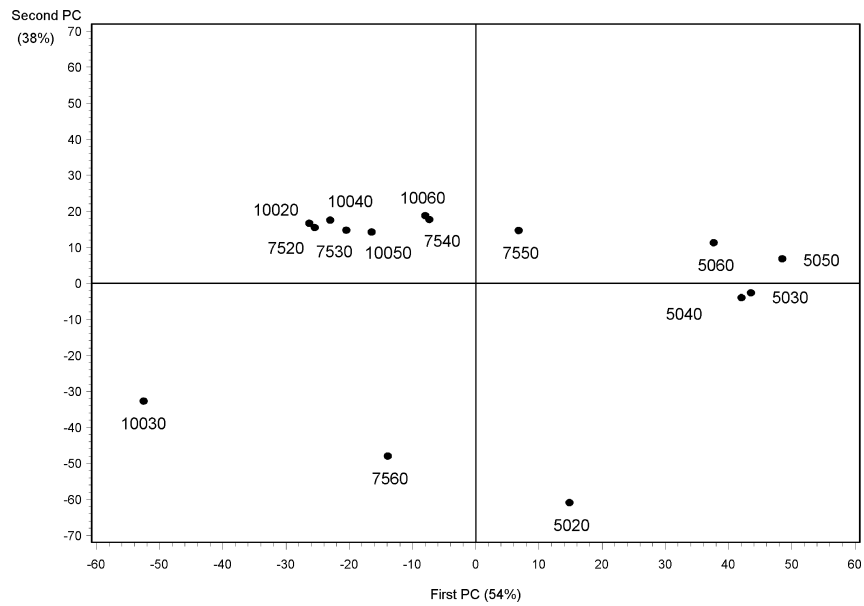

Figure 2—Spatial arrangement of process temperature and moisture for WPC, containing 80% protein based on the PCA of peak force of texturization (Component I, accounting for 54%), and foaming (Component II, accounting 38%). Code designation: 5030 (50 °C : 30% moisture); 10030 (100 °C : 30% moisture).

and 75 °C, the low solubility extrudates exhibited low to moderate foaming, while the 50 °C high solubility extrudates exhibited high foaming. The outlier from the group was the extrudate 75 °C/30% moisture, which was moderate foaming but of very weak forces. All products were of moderate to low foam expansion capacity. The exception was 75 °C/30% moisture, which were of moderate force and extremely low foam expansion capacity. We postulate that the higher the protein content in a whey product, the higher the degree of texturization above 75 °C, and that the higher the moisture content from 30% to 60%, the less texturization at a given temperature. Texturization can be hindered by other interfering whey components such as lactose. Proteins only are texturized, therefore, the higher the protein content the better texturization as measured by reduced solubility and increased WAI.

Discussion

The dairy products, NDM, WPC80, and WPI are substantially different in gross composition, particularly in lactose and protein contents, and therefore the degree of extrusion texturization for each protein product differed. However, as similarities in responses and trends can be correlated with the protein content, general trends dependent on preset process temperature and moisture are contrasted. Whey protein products, depending on their protein contents, reacted differently to extrusion texturization; therefore, the generalized effects of extrusion texturization were dependent on their effects on the proteins. The proteins are the components primarily affected by the changing temperature and moisture conditions that can most impact texturization. The degree of solubility of texturized whey proteins depends on the amount of protein present (Vojdani 1996). Li and Lee (1997) showed that the solubility of wheat gluten extruded at 120 °C corresponded with changes in protein ratio. High temperature unfolds proteins, affecting their stabilizing secondary and tertiary noncovalent bonds, and increases the interaction of sulfhydryl bonds, leading to increased hydrophobicity and aggregation (Pelegrine and Gasparetto 2005). Disulfide formation and formation of large aggregate structure increased with extrusion temperature (Li and Lee 1997). We have reported earlier that the rheological characteristics of extruded whey proteins can be manipulated to create desired properties (Tunick and Onwulata 2006). Manoi and Rizvi (2008) have confirmed these results, and have recently shown that extruding WPC80 impacts cold-setting properties (Manoi and Rizvi 2009).

Table 3—Physical properties of texturized WPI.

Temp (°C)	Water pump setting	Moisture (%)	Solubility (%)	WAI (%)	Foam_Exp	Peak_Force (N)	Prot_Dig	ΔE
50	20	43.8	92.6	6.9	214.3	48.9	87.7	88.6
50	30	53.8	84.6	8.8	212.1	54.2	85.5	88.5
50	40	60.9	79.3	8.0	176.4	10.9	59.4	94.7
50	50	65.1	77.5	7.3	174.3	1.5	89.8	89.2
50	60	67.6	71.8	7.1	205.7	0.3	88.5	88.7
75	20	38.4	3.0	30.2	124.3	129.8	87.1	85.5
75	30	46.7	11.6	18.9	180.0	199.3	90.8	90.1
75	40	58.4	18.5	24.0	160.0	121.8		85.7
75	50	62.6	21.3	27.9	175.8	8.6		89.7
75	60	66.1	21.4	27.5	144.3	2.7	86.1	89.2
100	20	34.4	3.6	36.4	123.6	147.1	88.9	85.3
100	30	42.7	4.3	31.2	134.2	102.6	89.4	88.4
100	40	53.8	7.3	28.9	172.8	54.9	87.9	88.3
100	50	53.7	5.5	29.1	142.1	81.9	91.5	78.6
	R^2	0.98	0.93	0.92	0.75	0.74		

Temp = temperature set points; Water = water set points; WAI = water absorption index; Foam_Exp = foam expansion; Prot_Dig = protein digestibility; and ΔE = color difference. Native protein solubility 98.4; R^2: Significant correlations ($P < 0.05$). Mean square error values were: Moisture (2.55), Solubility (17.5), WAI (0.10), Foam_Exp (420.2), Peak_Force (1853.4), Prot_Dig (0.57.3), and ΔE (11.8).

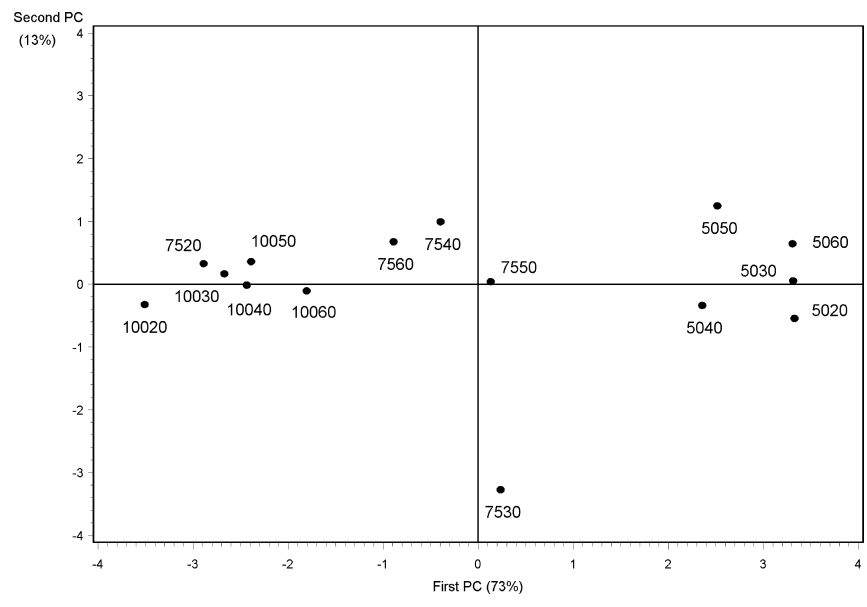

Figure 3—Spatial arrangement of process temperature and moisture for WPI, containing 95% protein, based on the PCA of peak force of texturization (Component I, accounting for 73%), and foaming (Component II, accounting 13%). Code designation: 5030 (50 °C : 30% moisture); 10030 (100 °C : 30% moisture).

The classical descriptors of texturized globular proteins are the disruption of the disulfide bonds, and the ability of the process to alter the secondary and tertiary substructures of a protein. The mechanism involves breakage of interactive forces, hydrogen bonds, -S—S- bonds, and forming physical linkages by thermal denaturation. Also, thermal denaturation involves thermo-reversible gelation, nonreversible gelatinization, and nonreversible aggregation or texturization (Kinsella 1978). The process of insolubilization or texturization is described principally as aggregation and crosslinking resulting from thermalization (Giddy 1983). Using insolubilization as a measure of texturization, our results show relative differences in gelatinization, especially with WPI extrudates, containing the most protein (> 95%). The solubility trends for the 3 whey products showed: little effect due to extrusion on NDM either by temperature or moisture (Table 1), and increasing solubility with moisture for both WPC80 (Table 2) and WPI (Table 3) as texturization occurred at 75 or 100 °C. Using the spatial arrangement, WPI extrudates separated in 3 temperature clusters (Figure 3). The 100 and 75 °C/20% moisture products were completely texturized with very low solubility, corresponding to nonreversible aggregation. The solubility of the nontexturized 50 °C products was not changed, showing minimal thermalization. Nontexturization is further confirmed by the low peak force for NDM, which were reduced further by increasing moisture. Peak force for WPC80 and WPI were significantly ($P < 0.05$) reduced at higher moisture, temperatures notwithstanding. Ordinarily, reduced water absorption can be an evidence of texturization, indicating that morphological changes of the proteins resulted in the loss of its innate ability to absorb water, as shown by Pelegrine and Gasparetto (2005), that solubility of whey proteins was altered by increasing temperature. Thermomechanical treatment of collagens resulted in increased molecular weight and solubility, without peptide bond cleavage. Previous studies show that solubilization of collagen by thermomechanical shear changed its internal fibrous structure without cleavage of peptide bonds, and that enhanced solubility of denatured collagen in water was within the temperature range of 50 to 95 °C (Meyer and others 2005). Texturization below 100 °C does not alter protein digestibility (Onwulata and others 2003). Solubility of proteins integrates the overall effect of extrusion texturization on its properties as the result of changes in structure and conformation. Relative to whey protein product type, texturization effects are in order NDM < WPC80 < WPI (Figure 4). On the aggregate, extrusion shear, not extrusion temperature texturized NDM. WPC80 and WPI were texturized at temperatures of 75 and 100 °C.

Microscopic imaging, scanning electron microscopy (SEM), and confocal scanning laser microscopy (CSLM) of texturized protein products (Figure 5 and 6, respectively), show differences in fine and macro structures dependent on whey product type. These structural differences can be seen in Figure 5, for NDM, 34% protein (5A), WPC80, 80% protein (5B), and WPI, 95% protein (5C) showing the effects of extrusion at 75 °C and 30% moisture. Protein aggregation is visible in WPI (5C) showing aggregation with folds ranging from 10 to 50 microns. Extruded NDM (5A) had a continuous lactose matrix with 1 to 3 micron gaps, and within the gaps are filamentous network stretches of texturized protein bodies. Stretched filaments were more pronounced in WPC80 (5B), which comprised of a mesh of nano-sized fine structures. Texturized WPI (5C) consisted of aggregates and networks masses of 2 to 10 micron folded proteins. Fracture faces of crosslinked, frozen, and critically point

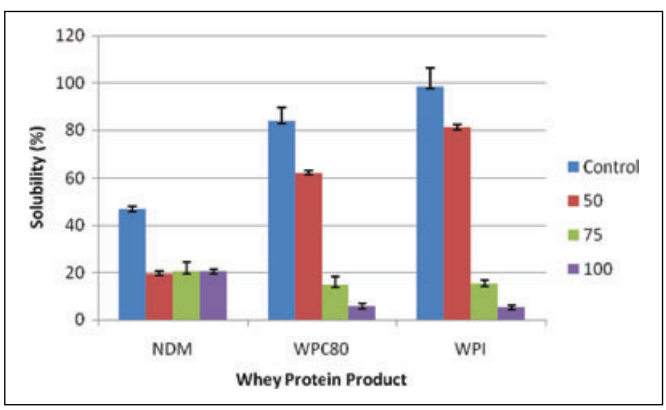

Figure 4—Average water solubility of NDM, WPC80, and WPI extruded at 50, 75, and 100 °C.

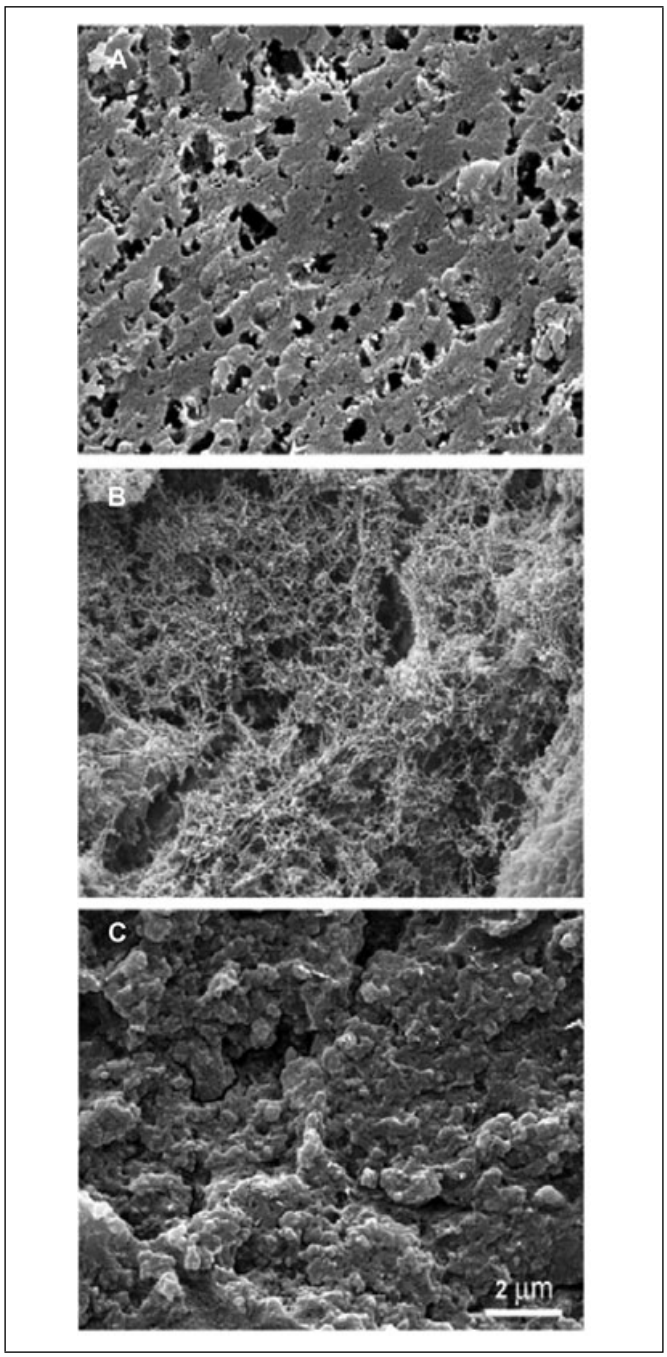

Figure 5—Scanning electron microscope images of NDM, 34% protein (Figure 4A), WPC80, 80% protein (Figure 4B), and WPI, 95% protein (Figure 4C).

dried strips of extruded ribbons were different for each type of sample (Figure 5). Some areas of NDM surface were porous with holes ranging from less than 0.5 to over 1 μm in diameter, and the continuous phase was composed of irregular areas around the pores with widths ranging from 1 to 2 μm (5A). Many fractured facial areas of WPI and WPC80 were filled with heaps of irregularly oriented networks of fine filaments composed of circular subunits, approximately 10 nanometers in diameter (5B). The WPI sample was composed of close-packed granules ranging from 100 nm to over 1 μm in diameter (5C).

The folding may have resulted from the hydrophobicity of texturized proteins. More details of the structures can be seen in Figure 6, where the green autofluorescence of texturized proteins from CSLM images for NDM (6A), WPC80 (6B), and WPI (6C) show strong aggregation for WPI (6C). Autofluorescence from the glutaraldehyde-crosslinked proteins in cross-sectioned strips of the extruded ribbons was visualized in maximum projection images made from stacks of consecutive serial optical sections ranging from 20 to 30 μm (Figure 6). Fluorescence in NDM samples was homogeneous on a microscale with minor, gradual changes in intensity between regions around 50 μm in diameter (6A). Fluorescence intensity in WPC80 samples was the highest and most uniform (6B) and contrasted sharply from the fluorescence in WPI samples, which was granular, polydisperse, and loosely clumped; the largest granules were over 50 μm in diameter and the smallest were less than 10 (6C). Generally, folded aggregated structures affected the peak forces as reported in Table 1, 2, and 3. The open structures with mesh-like networks formed at intermediate temperature (75 °C) and high moisture (40% to 60%) resulted in moderate peak forces. The nonaggregated structures were soluble and of weak peak forces. The solubility pattern agrees with the findings of Walkenstrom and others (1998) that difference in gel properties of WPI depended on shear rate and temperature, which affects aggregation and gel strength. This agrees with the findings of this study on texturization.

The variously heat treated samples were compared by SDS-PAGE (Figure 7). The SDS gels indicated some change in solubility. SDS gels developed initially without the reducing reagent 2-mercaptoethanol show the protein disulfide bonds to be intact

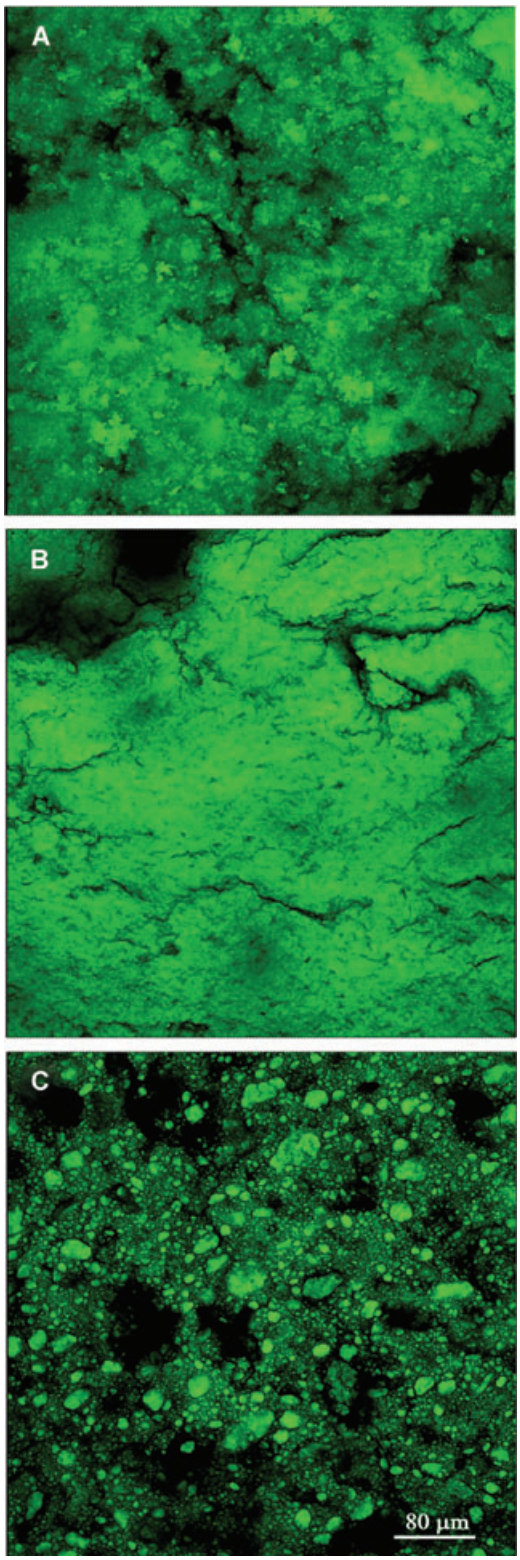

Figure 6—Confocal scanning laser microscope of NDM, 34% protein (Figure 5A), WPC80, 80% protein (Figure 5B), and WPI, 95% protein (Figure 5C).

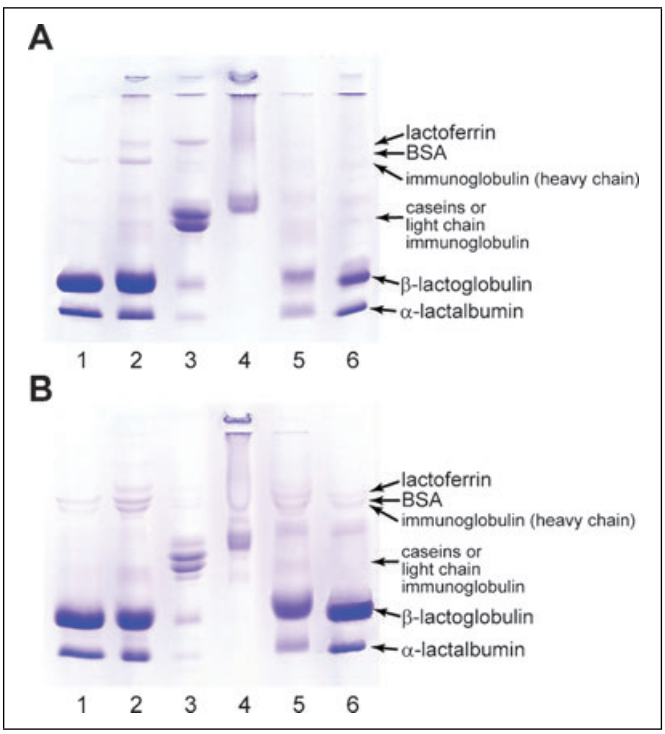

Figure 7—SDS PAGE Figure 7A and 7B—lane 1: NDM, lane 2: texturized NDM; lane 3: WPC80, lane 4: texturized WPC80; lane 5: WPI, and lane 6: texturized WPI.

with somewhat diminished bonds for the higher molecular weight (Figure 7A). At higher temperatures 75 and 100 °C the extruded whey samples were fainter, different from the native (nonextruded) whey on the SDS gel. In this respect, the SDS gels agree with the solubility data in that increased temperatures decrease solubility in SDS, suggesting sulfhydryl-disulfide crosslinking. The unreduced non extruded samples (7A) in lane 1 (WPI), lane 2 (WPC80), and lane 3 (NDM), show typical whey protein distribution patterns for WPI and WPC80, with more β-LG and α-LA than NDM. NDM had more casein and light chain immunoglobulins. With the extruded samples, lane 4 (NDM), lane 5 (WPC80), and lane 6 (WPI), the β-LG and α-LA protein bands became faint, and the light/heavy chain immunoglobulins became more pronounced. When the samples were reduced with 2−mecaptoethanol, disulfide bonds cleaved, and all the extruded whey samples at the different temperatures were similar to each other and to the lab-made (native) control whey (Figure 7B). The native and extruded whey products still have the same amount of the different proteins, thus texturization does not affect protein ratios. Others have reported that whey isolates were the most denatured by heat among different types of food proteins extruded (Mohammed and others 2000). In other proteins such as soy protein isolates and gluten, extrusion at high temperatures and low moisture results in structures with hard to break disulfide linkages (Prudencio-Ferreira and Areas 1993). In egg proteins as well, the degree of denaturation of egg proteins correlated with absorbed water based on conformational changes, and not by the presence of hydrophobic regions (Kumagai and others 1997). We have also observed that the denatured whey protein system behaved similarly to extruded soy protein and gluten, showing a similar pattern of bonding and crosslinking.

The viscous and elastic modulus of the whey products represented by their tan delta values, the ratio of elastic to loss modulus, is presented in Figure 8. The tan delta values of the NDM samples were in a narrow range and generally decreased as the pump setting increased. The values were all less than 0.3, indicating solid-like behavior. The whey products displayed different behaviors due to the differences in protein content. WPC80 and WPI, with 80% to 95% protein were comparable to each other. The moisture uptake in the samples at 50 °C resulted in samples that were nearly liquid-like. The tan delta values ranged from 0.20 and 0.27 for the NDM. Most of the 50 °C WPC and WPI samples were liquid-like, with loss modulus values greater than 0.5. The WPC80 and WPI samples extruded at 75 and 100° C were solid-like. These results of WPC80 and WPI reflect the increase in shear denaturation and texturization at these extrusion temperatures. Manoi and Rizvi (2008) have reported that texturized WPC80 possessed extremely high viscosity and forms gels at ambient temperature, while the commercial nonextruded WPC80 produced liquid dispersion.

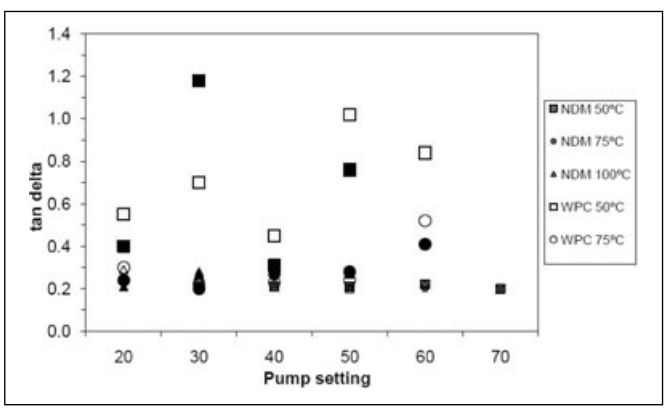

Figure 8—Loss tangent of NDM, WPC80, and WPI extruded at 50, 75, and 100 °C.

Conclusions

Adjustment of water addition and temperature led to differences in texture, moisture, and solubility of extruded milk proteins. The moisture content of extruded NDM, WPC, and WPI increased with water pump setting. Extruded NDM, nearly all of which is casein and lactose, remained solid within a relatively narrow range of G′ and loss modulus values. Extruded WPC and WPI, which contained 77% to 90% protein, liquefied when extruded at 50 °C, became soft at 75 °C and high moisture content, and were solid at 100 °C, reflecting the level of heat-induced and shear-induced denaturation. The range of physical properties exhibited by extruded milk powders will allow food processors and ingredient manufacturers to tailor their protein-fortified products according to their customers' needs. Extruded NDM, WPC, and WPI can all be used as food ingredients depending upon the properties desired and the type and amount of protein a manufacturer wishes to include. NDM produced a solid product with less moisture than the whey proteins when extruded at 50 or 75 °C, and with higher WSI when extruded at 50 °C. The solid-like nature of NDM is not because of texturization of proteins, but due to the abundance of crystalline lactose. Extruded WPI contained more moisture than the other extruded proteins, and was usually less solid-like. When extruded at 50 °C, the moisture content of WPC was close to that of NDM, but the WSI was lower and the product became fluid-like as water content increased. At the other temperatures, the WPC moisture content was lower than that of WPI and the WSI was higher than that of NDM. Whey proteins are now being included in a number of food products, and modification by extrusion may allow for wider applications and greater utilization.

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